REMARKS

Applicants request reconsideration of this application in view of the foregoing amendments and the comments that follow.

I. Status of Claims

New claims 39-40 are added with support at, for instance, last sentence on page 23 of the specification.

Amended claim 1 recites that the minicells are free of contamination from membrane blebs that are 200 nm or less in diameter. The specification supports this feature by virtue of disclosing, for example, in the last paragraph on page 23, that minicells "carrying … recombinant DNA are … purified by procedures ... described in PCT/IB02/04632," which provides that such purification entails "filtration through a 0.2 μm cross-flow filter" whereby "[b]acterial blebs [that] range in size (diameter) from 0.05 μm to 0.2 μm [necessarily] are filtered out" (page 41, lines 20-23).*

No impermissible new matter has thus been introduced. See MPEP § 21603.07(a) (disclosure in specification that necessarily conveys property or advantage supports claim amendment to recite same). Applicants therefore request entry of this amendment, whereupon claims 1, 3-7, 9, 12-18, and 36-40 will be pending.

II. Substance of Examiner Interview

Applicants' representative thanks Examiner Singh for the courtesy of a telephone interview on November 21, 2011. During the interview, the representative and the examiner discussed whether an additional feature, that the minicells are free of membrane blebs of 200 nm or less in size, would overcome the rejections. No agreement was reached.

* The international application, PCT/IB02/04632, was made of record with a Information Disclosure Statement filed June 7, 2005, which listed the document by its publication number, WO 2003/033519.

III. Response to Section 103 Rejection

The examiner has rejected all pending claims for alleged obviousness over Sabbadini, Grillot-Courvalin, Khatchatourians, Christen, and four other secondary references. Without acquiescing to this rejection, applicants have revised claim 1 further to recite that the minicells are free of contamination from membrane blebs 200 nm or less in diameter.

A. The Amended Claims

Claim 1 is directed to a gene delivery method that entails bringing bispecific ligands into contact with (a) intact bacterially derived minicells that contain a plurality of therapeutic nucleic acid sequences each operably linked to a promoter and (b) non-phagocytic mammalian cells. Claim 1 prescribes that the bispecific ligand has a specificity for a mammalian cell surface receptor that is capable of activating receptor-mediated endocytosis (rME). Claim 1 further states that the recited minicells are approximately 400 nm in diameter and are free of contamination from membrane blebs 200 nm or less in size.

Pursuant to the claimed methodology, the mammalian cells engulf the minicells by virtue of the ligand's specificity, and the cell expresses the therapeutic nucleic acid sequences. This uptake of minicells contravenes contemporaneous dogma to the effect that rME could not accommodate entry of particles with a diameter larger than 120 nm into non-phagocytic mammalian cells. So informed, the skilled artisan would have been directed away from an association between a bispecific ligand with the prescribed capability and a particle, the intact minicell, with a diameter several multiples larger than 120 nm.

This conventional wisdom notwithstanding, the present inventors discovered that intact, bacterially derived minicells unexpectedly are taken up by non-phagocytic mammalian cells through rME. It is only on the strength of these and other insights, not presaged by the art, that the inventors could establish that associating a bispecific ligand as prescribed with a minicell (about 400 nm in diameter) greatly enhances such uptake, leading to improved gene delivery efficiency.

B. The Rejection

Examiner Singh reads Sabbadini to "teach a method of gene delivery by covalently attaching binding moieties including antibody to minicells via membrane proteins that bind[] to a ligand present on the surface of a mammalian cell." Action at page 6, first paragraph. The examiner also acknowledges that Sabbadini' disclosure differs from the rejected claims by that Sabbadini does "not explicitly disclos[e] that minicells are 400nm in diameter." *Id.*

Yet, the examiner cites Khachatourians to teach removing contamination from patent bacterial cells and cites Christen to teach the use of 0.45 microns filter in purification of minicells. As such, the examiner is of the opinion that the cited art motivates the skilled artisan to "remove other potentially harmful contaminants as a matter of design choice to obtain more specific delivery of therapeutic agent [] to yield predictable results." *Id.* at page 7, first paragraph.

In sum, the examiner reads Sabbadini to disclose, for purposes of gene delivery, the combination of bispecific ligand with any from diverse "minicell" category that Sabbadini teaches, regardless of the size of the "minicell." The examiner relies on Khachatourians and Christen for teaching removal of parent cells that are larger than 0.45 micron.

Applicants respectfully traverse this rejection for at least the reasons that **(A)** the prior art would not have suggested that bacterial vesicles of 200 nm or less in diameter, such as membrane blebs, should be removed from the minicells and **(B)** conventional understanding actually disincentivized the removal of such small bacterial vesicles, particularly in the context of associating minicell with a bispecific ligand, as the pending claims prescribe.

C. The Prior Art Does Not Suggest Removal of Smaller Bacterial Vesicles Such as Membrane Blebs

C.1 The cited references do not suggest removal of membrane blebs

With respect to minicells, nothing in the cited art implicates removing membrane blebs or other small particles for any purpose, let alone for therapeutic uses. To the contrary, Sabbadini teaches that "minicells" can be sized anywhere from about 50, 100, 150, 200 to about 250, 300, 350,

400, 450 or 500 nm (col. 38, lines 30-31), and nothing is said about sizes that might be preferred over others.

As applicants' last response underscored, Sabbadini defines "minicell" to encompass a large genus of bacterial vesicles that includes membrane blebs, which are about 200 or less in size. See response filed September 1, 2010, *e.g.*, at section 2.2 on pages 17 and 18. Thus, the primary reference does not disclose but rather teaches away from a minicell population that is free of contamination from membrane blebs that are 200 nm or less in size.

Moreover, Khachatourians and Christen relate only the removal of parental cells, which are larger than derived minicells. There is not a hint regarding removal of particles that are smaller than the ~400-nm diameter of intact, bacterially derived minicells.

Applicants therefore submit that the record does not substantiate the allegation of a *prima facie* case of obviousness. Consequently, withdrawal of the subject rejection is warranted.

C.2 The References Cited in Related Applications Do Not Suggest Removal of Membrane Blebs

Applicants concurrently submit a Supplemental Information Disclosure Statement that includes Kadurugamuwa and Beveridge *J. Bacteriol.* 177: 3998-4008 (1995), a document cited in an Office action, also submitted, from co-pending, commonly owned application serial No. 12/019,090. Commentary in the Office action addresses the Sabbadini reference of record (see pages 13 – 15) and, in that context, extrapolates from Kadurugamuwa with respect to "bleb" contamination.

Thus, "[r]egarding the production of bacterial blebs from … minicell producing parent bacterial cells," the Office action states"

> … the art teaches that bacterial blebs are of a known size and potentially pathogenic in a host organism. Specifically, Kadurugamuwa et al. … teaches that parent bacterial cells naturally produce blebs (aka membrane vesicles or MV's) which are 50 to 150 nm in size (pg. 3998, Abstract lines 1-5). Kadurugamuwa continues to teach that MV's such as blebs "could thus play an important role in the genetic transformation of disease by serving as a transport vehicle for DNA and virulence factors and are presumably involved in septic shock" (Abstract, last sentence).

> The ordinary artisan, given the teachings in the art, would know and be distinctly motivated to remove contaminants 0.2 μm or less in size from a minicell preparation taught by Sabbadini since it was known at the time of filing that Sabbadini's minicells were predominantly 400 nm in size and that bacterial blebs produced during the minicell production process were in the 50-150 nm size range and through their presence could lead to potential health consequences if not filtered out from the produced minicells.

Page 14, last paragraph – page 15, first full paragraph.

As explained on pages 31 and 32 of the last response, however, the prior art illustrated by Sabbadini, Hale, and Jaffe evidenced no inkling of a particular size, as opposed to a broad size range, for intact, bacterially derived minicells. In a related vein, the foregoing characterization of the Kadurugamuwa publication errs in ascribing to the authors the notion that membrane blebs (*a/k/a* "membrane vesicles") as a class "could lead to potential health consequences" and, hence, would have been "filtered out" from a minicell preparation.

To the contrary, Kadurugamuwa is explicit in emphasizing virulence factors, along with DNA, LPS and other factors produced by the parent bacterium, that are encased in membrane vesicles and released from the parent cell. It would have been apparent, therefore, that these factors and not the membrane blebs *per se* contribute to diseases such as inflammation and septic shock.

The knowledgeable reader of Kadurugamuwa would have understood, therefore, that virulence factors in *either* a virulent parent bacterium *or* in its shed membrane vesicles (membrane blebs) are the cause of pathogenesis. Indeed, the use of a virulent bacterium would fairly ensure that intact minicell derived from the bacterium likewise would contain the same virulence factors, in keeping with Kadurugamuwa.

Thus, the skilled artisan would have gleaned from the Kadurugamuwa publication that <u>both</u> minicells <u>and</u> membrane blebs from a virulent bacterial strain contain the virulence factors of that strain; hence, that neither minicells nor blebs from the virulent strain should be retained. In other words, Kadurugamuwa suggests <u>no difference between minicells and membrane blebs</u> in terms of safety. If anything, therefore, the person of ordinary skilled would have been *disincentivized*, not

motivated, to remove contaminants in the size range of membrane blebs (200 nm or less) from a minicell preparation, *i.e.*, precisely the opposite to what the listed action posits, incorrectly.

No prior art of record in this application or in applicants' related cases teaches or suggests the removal of small particles, such as membrane blebs, from a minicell preparation.

D. The Conventional Wisdom Disincentivized the Removal of Smaller Bacterial Vesicles Including Membrane Blebs

D.1 The conventional wisdom recognized dogma that particles with a diameter larger than 120 nm cannot enter cells by receptor-medicated endocytosis

Applicants' claimed methodology employs (a) minicells of about 400 nm in diameter and (b) bispecific ligands that can bind a mammalian cell surface receptor to induce rME and consequent minicell uptake. To arrive at this combination of (a) and (b), applicants submit, the skilled artisan would have had to understand that a target mammalian cell, albeit non-phagocytic, can uptake minicells via rME even though the minicells are about 400 nm in diameter. Prior to the filing of the present application, however, conventional wisdom taught exactly the opposite.

Applicants explained in their last response (pages 8-10) that large particles enter a host cell through an active invasion or a passive endocytosis process. When entering through active invasion, the particle (*e.g.*, a bacterium) invokes a mechanism, such as the use of invasins (*id.* at page 10), to ***avoid*** being degraded and releasing its nucleic acid content.

A particle capable of active invasion thus cannot be used for gene delivery, which ***requires*** release of a nucleic acid into the host cell. For the skilled artisan, therefore, this principle would leave only passive endocytosis, or more particularly, rME, would be the only avenue available, even in theory, for gene delivery in this context. As noted previously, however, it was widely accepted that particles larger than ***120 nm*** could not enter cells by rME. See last response at page 11, second and third full paragraphs.

With this conventional perspective, the skilled artisan would have approached choosing from of selecting a "minicell" from Sabbadini's broad genus with, if anything, a bias toward particle sizes

substantially smaller than ~400 nm. Thus, on one hand the person of ordinary skill would have been motivated to remove "minicells" that were much larger than the perceived upper size limit of rME, such as bacterially derived minicells, for the purpose of exploiting the rME-activating capability of a bispecific ligand. On the other hand, the skilled artisan would have been disincentivized from removing membrane blebs, given their size below the perceived rME limit.

D.2 Commentary about Grillot-Courvalin in the context of bacterial cell-mediated gene delivery

In light of the above-discussed dogma and its ramifications for the understanding of the skilled artisan, applicants would like to comment on Examiner Singh's interpretation of Grillot-Courvalin. According to the examiner, Grillot-Courvalin teaches that, upon co-incubation with host cells, bacterial cells release plasmid DNA that is expressed in the host cell. Action at page 4, last paragraph.

This interpretation of Grillot-Courvalin is incomplete, however, and thus is in error. The reference discusses an "abortive and suicidal **invasion**" process by which a bacterium enters a host cell (abstract; emphasis added). In contrast, the non-living minicells recited in applicants' claims are by nature incapable of entering a host cell through invasion. Furthermore, Grillot-Courvalin disclosures in no way detract from or modulate the contemporaneous dogma concerning an rME size limit, which, as noted above, cuts against the combination of applicants' claimed invention.

In summary, therefore, the prior art does not teach or suggest that bacterial vesicles that are 200 nm or less in diameter, such as membrane blebs, should be separated from the minicells and removed, and conventional knowledge further disincentivized such removal. Accordingly, the examiner cannot establish a sustainable *prima facie* case of obviousness on the prior art of record. Withdrawal of the Section 103 rejection is solicited, therefore.

CONCLUSION

Applicants submit that this application is in condition for allowance, and they request and early indication to this effect. Examiner Singh is invited to contact the undersigned directly, should he feel that any issue warrants further consideration.

Respectfully submitted,

Date February 17, 2012

By /Stephen A. Bent/

FOLEY & LARDNER LLP
Customer Number: 22428
Telephone: (202) 672-5404
Facsimile: (202) 672-5399

Stephen A. Bent
Attorney for Applicants
Registration No. 29,768

The Commissioner is hereby authorized to charge any additional fees, which may be required under 37 C.F.R. §§ 1.16-1.17, and to credit any overpayment to Deposit Account No. 19-0741. Should no proper payment accompany this response, then the Commissioner is authorized to charge the unpaid amount to the same deposit account. If any extension is needed for timely acceptance of submitted papers, then applicants hereby petition for such extension under 37 C.F.R. §1.136 and authorize payment of the relevant fee(s) from the deposit account.